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**An association study between two missense variations of the
benzodiazepine receptor (peripheral) gene and schizophrenia in
a Japanese sample**

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Summary. The benzodiazepine receptor (peripheral) (BZRP) mainly localized on glial cells plays a role in neurosteroid synthesis, and increases with glial proliferation. We have recently reported a significant decrease in the density of BZRP labeled by [³H] PK 11195 in the postmortem brain of chronic schizophrenics, suggesting that dysfunctions of the BZRP are involved in the pathophysiology of schizophrenia. We screened 11 patients with schizophrenia and 10 controls, which were used in a previous postmortem study, for their genomic sequences of the BZRP gene in order to find DNA sequence variations. One novel missense polymorphism (His162Arg) and another previously reported missense mutation (Ala147Thr) were detected. An association study of the identified variations was then performed in an extended Japanese sample of 304 schizophrenic patients and 369 controls. While there was an increased tendency in the frequency of the 162Arg allele of schizophrenics compared to that of the controls ($p = 0.0603$), no statistically significant association with schizophrenia was observed in the Ala147Thr allele ($p = 0.1016$). These results do not suggest that the two missense polymorphisms play a major role in the genetic predisposition of schizophrenia in the Japanese sample.

Keywords: Benzodiazepine receptor (peripheral), schizophrenia, single nucleotide polymorphism, association, missense, restriction fragment length polymorphism.

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Introduction

The benzodiazepine receptor (peripheral) (BZRP) is distributed in the peripheral tissues, such as the adrenal gland and testis, as well as in the central nervous system (De Souza et al., 1985; Kurumaji and Toru, 1996). The receptor plays an important role in the rate-limiting step of steroidogenesis, which localizes in the mitochondrial outer membrane of steroid-producing cells (Kreuger and Papadopoulos, 1990). The BZRP mainly localized on astroglial cells (Itzha et al., 1993) is also involved in steroid genesis in the brain mitochondrial preparation (McCauley et al., 1995; Papadopoulos et al., 1992). The neurosteroids putatively elicit potent short-term allosteric modulatory effects on the action of GABA at the GABA_A receptor (Majewska, 1992), which may produce an anxiolytic effect (Auta et al., 1993), and long-term genomic-mediated effects acting at the intracellular steroid receptor (McEwen, 1991).

BZRP has been shown to be increased in the brain following ischaemic (Stephenson et al., 1995) and excitotoxic neuronal damages (Benavides et al., 1987), and some neurodegenerative diseases such as Huntington's disease (Meßmer and Reynolds, 1998; Schoemaker et al., 1983). We have reported that the density of BZRP labeled by [³H] PK 11195 in the postmortem brains of chronic schizophrenic patients was decreased in three brain areas (superior parietal cortex, primary visual area and putamen) within 26 examined brain regions, and that the maximum number of binding sites (B_{max}) as well as the dissociation equilibrium constant (K_d) were reduced in the brain areas of schizophrenics (Kurumaji et al., 1997). We have speculated that the reduced density of BZRP in the postmortem brains of schizophrenics may be involved in the pathophysiology of schizophrenia, associated with dysfunctions of neurosteroids, and that the decreases in both B_{max} and K_d might be attributed to unknown alterations in the conformation and/or structure of BZRP in schizophrenia (Kurumaji et al., 1997).

The BZRP gene, localized in the 22q13.3 band of the human genome (Riond et al., 1991), consists of four exons, with exon 1 encoding only a short 5' untranslated segment (Lin et al., 1993). Several studies have reported some evidence for linkage between schizophrenia and genetic markers located on chromosome 22q (Coon et al., 1994; Schizophrenia Collaborative Linkage Group for Chromosome 22, 1998). In the present study, we screened the coding sequence of the BZRP gene from exon 2 to exon 4 in 11 patients with schizophrenia and 10 controls used in the postmortem brain study (Kurumaji et al., 1997) to find variations resulting in a significant change in the BZRP functions. The two missense polymorphisms of the nucleotide sequence in exon 4 was detected, one of which is a novel one. Furthermore, we conducted an association study between schizophrenia and these missense polymorphisms.

Materials and methods

Subjects

The schizophrenics examined in this study were 304 unrelated Japanese patients (175 males and 129 females) with a mean \pm S.D. age of 44.5 ± 12.5 , including 11 patients used

in the previous postmortem brain study (Kurumaji et al., 1997). All patients met the criteria for schizophrenia of the American Psychiatric Association's Diagnosis and Statistical Manual of Mental Disorders (DSM-III-R) (1987). The patients were receiving treatment at eight hospitals within 200 km of Tokyo. The control subjects were 369 unrelated Japanese (199 males and 170 females) including 10 controls used in the previous study (Kurumaji et al., 1997) with a mean \pm S.D. age of 45.5 ± 12.5 . Among the controls, 203 were paramedical staff members documented to be free of psychosis. The remainder were corporate employees who had requested annual physical examinations but had not been evaluated for psychiatric disorders by a psychiatrist.

This study was approved by the Ethics Committee of the Tokyo Medical and Dental University. Informed consent was obtained prior to the study.

DNA analysis

Genomic DNAs from all subjects were prepared from peripheral whole blood cells and postmortem brains by the phenol extraction method or by using a DNA Extraction Kit (STRATAGENE). The fragments including exons 2, 3 and 4 were amplified by the touch down polymerase chain reaction (PCR) using the primers and PCR conditions shown in Table 1. The PCR reaction mixture contained a total volume of 60 μ l consisting of 100 ng genomic DNA, 200 μ M of each dNTP, 5 μ M primers, 1.5 units of TakaRa Ex Taq (TAKARA SHUZO) in an equipped buffer. The "touchdown" PCR was done as follows: After denaturing at 95°C for 1 min, 15 sec at 94°C, 30 sec at T^* plus 11 minus N^{**} °C (T^* is listed in Table 1 and N^{**} is cycle number) and 1 min at 72°C for the first 10 cycles, and 10 sec at 94°C, 30 sec at T^* °C and 1 min at 72°C in the subsequent 20 cycles. Final elongation was 5 min at 72°C. Direct sequencing was carried out using the Big Dye terminator (PE Applied Biosystems, Norwalk, CT) on an ABI 377 auto sequencer (PE Applied Biosystems, Norwalk, CT).

Genotyping of the DNA sequence variants was performed by means of PCR-based restriction fragment length polymorphism (RFLP) assays (Table 2). A 30- μ l aliquot of the PCR product was incubated with 2 U of Nru I or 2 U of Nla III according to the manufacturer's recommendations (New England Biolabs, Schwalbach, Germany). Fragments were separated in a 3% Nusieve:Seakem agarose gel, and the bands were visualized by ethidium bromide staining and ultraviolet transillumination.

Statistical procedures

Deviation of the genotype counts from Hardy-Weinberg equilibrium were tested with HWE using Linkage Utility Programs (Lathrop et al., 1984). Differences between the patient with schizophrenia and the controls in allele frequencies and in genotype distributions were analyzed by the χ^2 test and by the Monte Carlo method using the CLUMP program (Sham and Curtis, 1995), respectively.

Results

The BZRP gene consists of four exons. Exon 1 encodes a short 5' untranslated segment, and exons 2, 3 and 4 code 169 amino acids of the BZRP protein. We screened 11 schizophrenics patients and 10 controls used in the previous postmortem brain study (Kurumaji et al., 1997) for sequence variations within the coding region (exons 2-4) using the direct sequence method. One novel missense variant in exon 4, which is derived from the nucleotide transition in codon 162 (CGT to CAT) resulting in the histidine to arginine (His162Arg) change (Fig. 1), was detected in three of 11 schizophrenics and 4 of 10 controls. Another missense variant in exon 4, which was previously reported (Mirel DM, accession ID of Genome Data Bank is 9861863), was also found

DDBS - DNA database of Japan
NCBI / GenBank

Table 1. PCR primers used to determine the sequences from exon 2, exon 3 and exon 4 and the polymorphisms in exon 4

Region	Primer name	Primer sequences	3' end of primer position	Annealing Temperature (T)* (°C)	PCR product (bp)
Exon 2	Exon 2 F	5'-CCC TCA CGC AGC CCT GTC TTC-3'	9bp upstream to exon 2		
	Exon 2 R	5'-CTT ATC CCC AGG CCA GTG CAC-3'	11bp downstream to exon 2	62	271
Exon 3	Exon 3 F	5'-TCC TAA TGG TGC TCT GAA CTG-3'	16bp upstream to exon 3		
	Exon 3 R	5'-GAT CAG GGA CAC ATG CTG TGG-3'	11bp downstream to exon 3	57	206
Exon 4	Exon 4 F	5'-TGG GAC AGG CAC TTG GGT GAA C-3'	45bp upstream to exon 4		
	Exon 4 R-1	5'-AAG CGT GAC GGC CAC CAC ATC A-3'	242bp downstream to 5'end of exon 4	63	329
	Exon 4 R-2	5'-ATG GCA CCT GCT GGT GCA GCT-3'	213bp downstream to 5'end of exon 4	62	299

The primers were designated from the sequences deposited in the Genbank data base (Accession nos. L21952, L21953, L21954)
* Final annealing temperature in the touch down method (see text)

Table 2. Detection of DNA sequence variants in exon 4 of human benzodiazepine receptor (peripheral) gene

Variant	Primer pair	PCR product (bp)	Restriction enzyme	Allele	Fragment size (bp)
<u>C</u> G/ <u>A</u> G at codon 162	Exon 4F & Exon 4R-1	329	Nla III	<u>C</u> GG	106 + 223
				<u>C</u> AG	9 + 97 + 223
<u>G</u> / <u>A</u> CG at codon 147	Exon 4F & Exon 4R-2	299	Nru I	<u>G</u> CG	115 + 184
				<u>A</u> CG	299

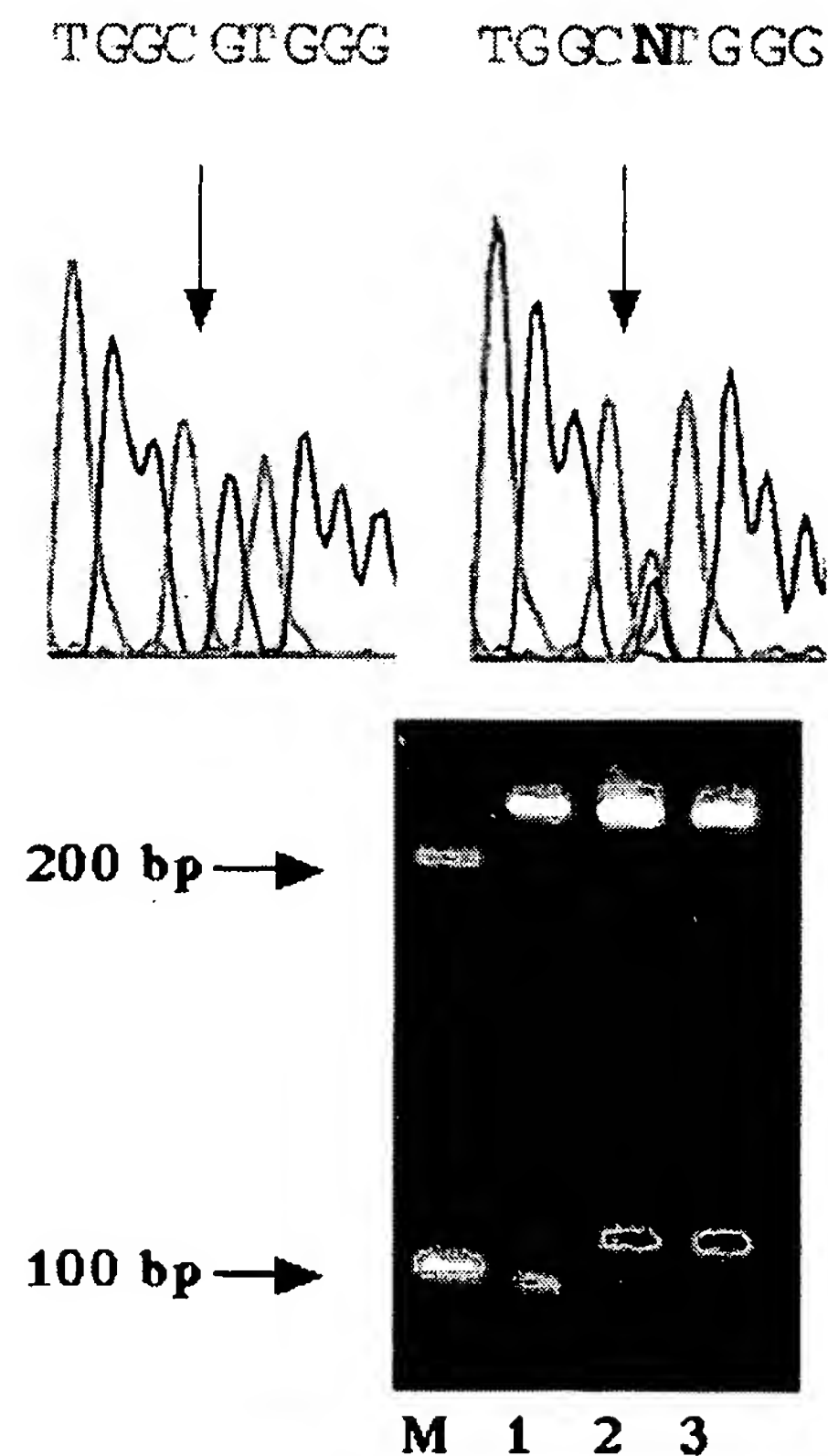


Fig. 1. Sequence and PCR-RFLP pattern of the His162Arg polymorphism. The PCR product amplified using the Exon 4F and Exon 4R-1 primers (Table 1) was digested with two units of restriction enzyme Nla III. The fragments were separated on 3% agarose gels. Lane 1, individual homozygous for CGG (His162His); lane 2, individual heterozygous for CG/AG (His162Arg); lane 3, homozygous for CAG (Arg162Arg); lane M, 100bp DNA Ladder (GIBCO BRL)

in one of 10 control subjects, which is a change from GCG to ACG at codon 147 with a substitution of alanine with threonine (Ala147Thr, Fig. 2). Although we further screened exon 2, exon 3 and exon 4 in an additional 40 schizophrenic patients by sequencing, no variation in the DNA sequences was observed.

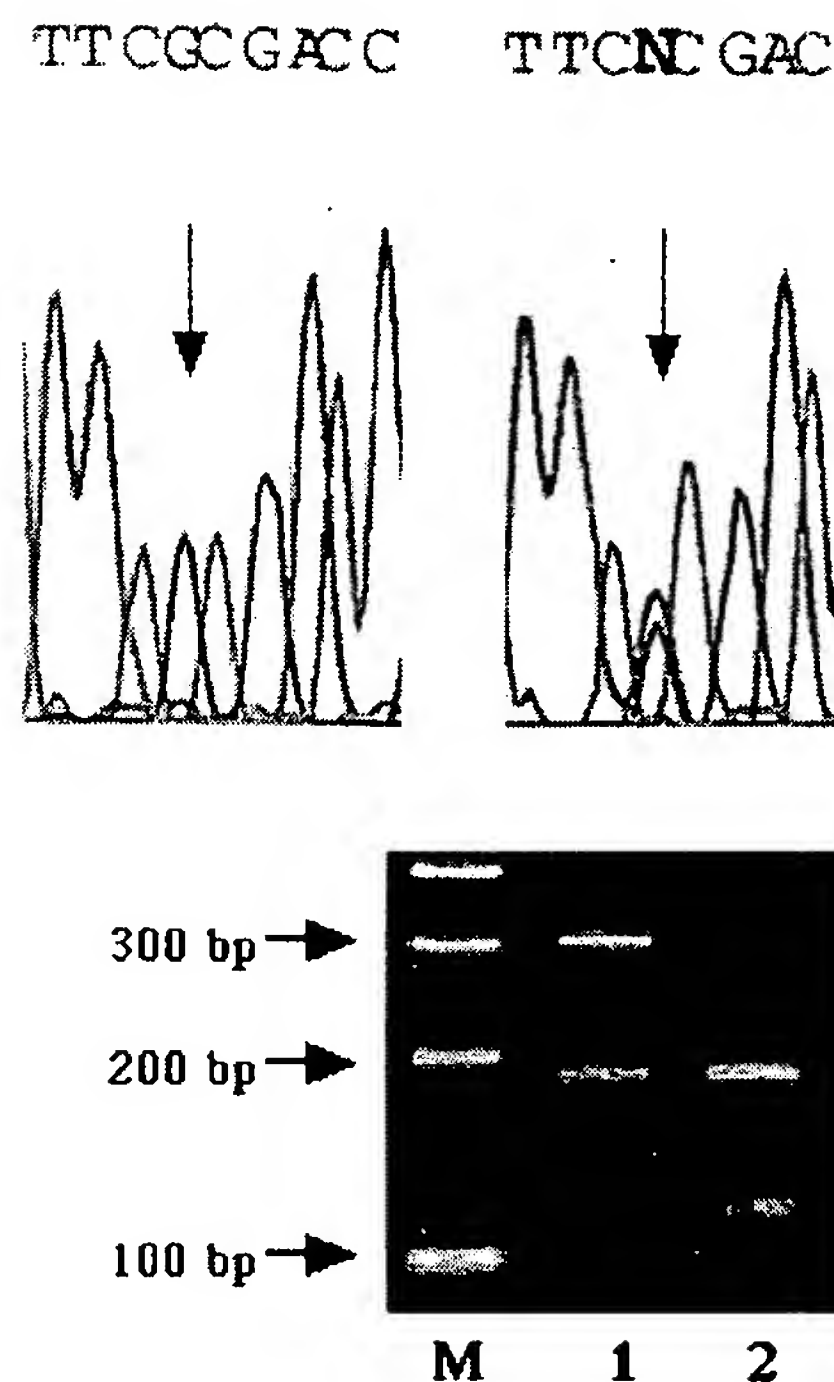


Fig. 2. Sequence and PCR-RFLP pattern of the Ala147Thr polymorphism. The PCR product amplified using the Exon 4F and Exon 4R-2 primers (Table 1) was digested with two units of restriction enzyme Nru I. The fragments were separated on 3% agarose gels. Lane 1, individual heterozygous for G/ACG (Ala147Thr); lane 2, homozygous for GCG (Ala147Ala); lane M, 100 bp DNA Ladder (GIBCO BRL)

The genotype and allele distributions of His162Arg in the schizophrenics and controls are shown in Table 3. There was no statistically significant difference between the schizophrenics and controls either in genotype ($\chi^2 = 4.323$, d.f. = 2, $p = 0.120$) or allele frequencies ($\chi^2 = 3.349$, d.f. = 1, $p = 0.060$) of the His162Arg variant. The frequencies of the 162Arg allele were 25.7% in the schizophrenic patients and 21.2% in the controls. Moreover, no statistically significant difference between the schizophrenics and controls was observed in the distribution of the genotype ($\chi^2 = 3.185$, d.f. = 2, $p = 0.103$) or allele ($\chi^2 = 2.543$, d.f. = 1, $p = 0.102$) of Ala147Thr (Table 4). The frequency of individuals carrying 147Thr was 3.8% for the schizophrenic group and 2.2% for the control group.

The distribution of genotypes in each variant did not significantly differ from the value expected according to the Hardy-Weinberg equilibrium: His162Arg [controls ($\chi^2 = 0.704$, $p = 0.4013$), schizophrenics ($\chi^2 = 3.269$, $p = 0.0706$)], Ala147Thr (controls ($\chi^2 = 0.181$, $p = 0.6703$), schizophrenics ($\chi^2 = 0.470$, $p = 0.4930$)).

Table 3. Allele frequency and genotype count for the His162Arg polymorphism of the benzodiazepine receptor (peripheral) gene in the controls and schizophrenics

Phenotype	Genotype count				p
	n	162Arg/Arg	162His/Arg	162His/His	
Controls	369	14	129	226	0.120 ^a
Schizophrenics	304	14	128	162	
	Allele count				
	n	162Arg		162His	
Controls	738	157		581	0.0603 ^b
Schizophrenics	608	156		452	

^a $\chi^2 = 4.323$, d.f. = 2, ^b $\chi^2 = 3.349$, d.f. = 1

Table 4. Allele frequency and genotype count for the Ala147Thr polymorphism of the benzodiazepine receptor (peripheral) gene in the controls and schizophrenics

Phenotype	Genotype count				p
	n	147Thr/Thr	147Ala/Thr	147Ala/Ala	
Controls	369	0	16	353	0.103 ^a
Schizophrenics	304	0	23	281	
	Allele count				
	n	147Thr		147Ala	
Controls	738	16		722	0.1016 ^b
Schizophrenics	608	23		585	

^a $\chi^2 = 3.185$, d.f. = 2, ^b $\chi^2 = 2.543$, d.f. = 1

Discussion

The present study revealed a novel missense polymorphism (His162Arg), and confirmed the previously reported missense variation (Ala147Thr) in exon 4 of the BZRP gene. Although there was a tendency of increased frequency of the 162Arg allele in the schizophrenic patients compared to that of the controls, no statistically significant difference between the schizophrenics and controls was detected in the distribution of the genotype or allele of both variation sites. These results suggest that the polymorphisms do not play a major role in the genetic predisposition of schizophrenia in the Japanese cohort.

The BZRP shows an ubiquitous distribution in the central nervous system and in the peripheral tissues (De Souza et al., 1985; Kurumaji et al., 1996). The receptor localizes in the mitochondrial outer membrane of the steroid-producing cells (Anholt et al., 1986; Antkiewicz-Michaluk et al., 1988), and plays an important role in the translocation of cholesterol from the outer to inner mitochondrial membranes, a rate-limiting step in steroidogenesis (Kreuger and Papadopoulos, 1990). The cDNA of the BZRP gene encodes a 18kDa protein which has five potential transmembrane regions (Sprengel et al., 1989). It is suggested that the 147Ala situates in the fifth putative transmembrane region, and that the 162His localizes in the carboxyl-terminal sequence. The 147Ala is conserved in BZRP of human, bovine and rodents, while the 162His is replaced by Arg in the receptor of bovine and rodents (Farges et al., 1994; Lin et al., 1993). Moreover, it is proposed that BZRP may conform a multimeric receptor complex composed of multiple 18kDa protein subunits and other proteins such as the voltage-dependent anion channel (Joseph-Liauzun et al., 1997; Papadopoulos, 1998).

The BZRP can be labeled with isoquinoline carboxamides such as PK 11195 and with benzodiazepines such as Ro5-4864, although the binding domains of the compounds are overlapping but not identical sites on the BZRP (Farges et al., 1993; Rao and Butterworth, 1997). The deletion of the residue 15–35 sequence of the amino-terminal of the BZRP resulted in the complete loss of binding of both [³H] PK 11195 and [³H] Ro5-4864, whereas the deletion of the last 14 amino acids of the receptor at its carboxyl terminus abolished the binding of Ro5-4864, with no effect on the binding of PK 11195. A particular residue of the sequence, 154Val, was crucial for the binding of the benzodiazepine ligand, but not of the isoquinoline ligand (Farges et al., 1994). In addition, the His162Arg and Ala147Thr variations determined in the subjects used in the postmortem brain study were not correlated to the changes in the binding parameters of [³H] PK 11195 in the brains (Kurumaji et al., 1997) (data not shown).

In conclusion, there were no associations between the two missense polymorphisms of the BZRP gene and schizophrenia in the Japanese cohort. Taking into account of the heterogeneity of schizophrenia, it may be important to investigate associations between these polymorphisms and schizophrenia in other populations. Moreover, it remains to be clarified whether or not the variations in the amino acid sequence of BZRP result in an altered interaction between BZRP and other mitochondrial membrane proteins and in a change of the physiological functions of the receptor complex. A search for other variations in the BZRP gene is also needed, particularly in the regulatory region, which can produce a change in the density of BZRP in the schizophrenic brains.

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Clone Id: WHE1405-1408_J03_J03
DNA type: cDNA

PRIMERS

Sequencing: Stratagene SK primer
PolyA Tail: Unknown

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Lib Name: Wheat drought stressed leaf cDNA library
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Cultivar: TAM W-101
Tissue type: Leaf
Develop. stage: Full tillering stage
Lab host: E. coli SOLR
Vector: Lambda Uni-ZAP XR, excised phagemid
R. Site 1: EcoRI
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Description: Plants were given a gradual stress down to 65% and 78% RWC at Texas Tech University (D. Zhang in HT Nguyen lab). Total RNA and poly(A) RNA were prepared, a cDNA library was made, and the cDNA clones were in vivo excised to give pBluescript phagemids in the TJ Close lab (Choi, Close), at the University of California, Riverside. Plasmid DNA preparations and DNA sequencing were performed in the OD Anderson lab (all other authors).

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- Try substring matching
If you gained no results or too few results, you can try substring matching. You can specify tex* to search for terms which begin with tex (e.g., tex, tex2html, texindex, text).
You can specify *tex to search for terms which terminated with tex (e.g., bibtex, jstex, latex, platex, ptex, vertex).
You can specify *tex* to search for terms which contain tex (many).
- You tried phrase searching but it hit documents which didn't contain your phrase.
It's a defect of Namazu. Precision of phrase searching is not 100 %, so it cause wrong results occasionally.
- You want to use and, or or not as ordinary keywords
You can surround them respectively with double quotes like "and" or braces like {and}

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Genomic Segment Query Results:

Nothing found

There are no Genomic Segments in the database which satisfy this query. It may help to make the query less restrictive by using general terms instead of specific terms or by filling in fewer fields. Here is a [list of the number of objects in the database](#). For additional help in formulating queries, send mail to help@gdb.org or phone 410-955-9705.

